Molecular diagnosis of haemophilia A: four novel variants identified in five patients

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Introduction:

Haemophilia A (HMA) is an X-linked bleeding disorder caused by reduced levels of the coagulation factor VIII (FVIII) due to alterations in the *F8* gene. Decreased levels of FVIII coagulant activity (FVIII:C) leads to a loss of clotting activity and consequent bleeding (predominantly into joins, muscles and inner organs). The severity of HMA ranges from mild (5-30% FVIII:C) to moderate (2-5% FVIII:C) to severe (<1% FVIII:C). During the last five years, we have found four novel variants identified in five index patients with no family history of HMA.

Results:

***F8* variant analysis allowed identification of three frameshift and one missense variants: c.1060_1061delCT, c.3561dupT and c.4804delC detected in families presenting severe HMA; c.5836G>T variant was identified in two unrelated patients with a mild phenotype (Table 1; Figures 1-4). None of these variants had been previously reported.**

Methodology:

Analysis of the *F8* gene was performed in five index patients (one female from a family without previous molecular studies, index case not available), using genomic DNA extracted from peripheral EDTA blood samples and specific PCR for *F8* exons, followed by Sanger sequencing. *F8* IVS22 and IVS1 inversions were excluded in severe HMA cases. Bioinformatics analysis was performed with several pathogenicity prediction tools (Alamut Visual, VarSome, VEP and Human Splicing Finder).

Table 1. Novel *F8* variants identified.

Patient	Variant type	HMA clinical phenotype	*F8 location*	Nucleotide change (NM_000132.3)	Amino acid change (NP_000123.1)
P1	Missense	Mild	Exon 18	c.5836G>T (Fig. 1)	p.(Asp1946Tyr)
P2					
P3	Frameshift	Severe	Exon 8	c.1060_1061delCT (Fig.2)	p.(Leu354Thrfs*5)
P4			Exon 14	c.3561dupT (Fig.3)	p.(Pro1188Serfs*10)
P5				c.4804delC (Fig.4)	p.(Gln1602Lysfs*19)

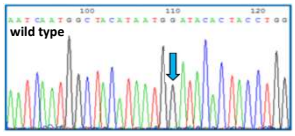


Figure 1. Electropherogram obtained from direct nucleotide sequencing of *F8* exon 18 (forward primer) showing *F8* c.5836G>T p.(Asp1946Tyr) variant.

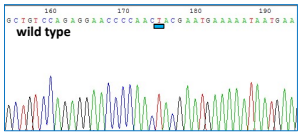


Figure 2. Electropherogram obtained from direct nucleotide sequencing of *F8* exon 8 (forward primer) showing *F8* c.1060_1061delCT p.(Leu354Thrfs*5) variant. Results from a female carrier.

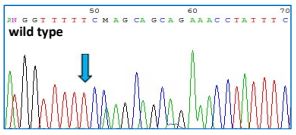


Figure 3. Electropherogram obtained from direct nucleotide sequencing of *F8* exon 14 (forward primer) showing *F8 c.3561dupT p.(Pro1188Serfs*10)* variant.

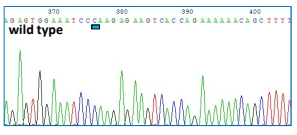
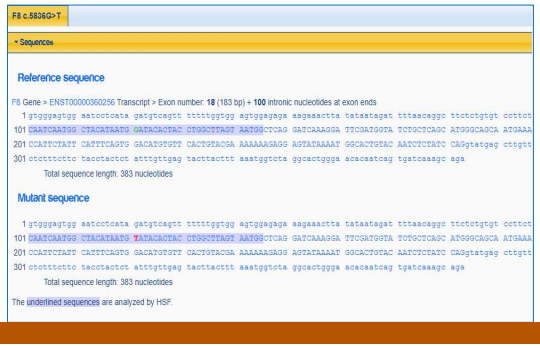
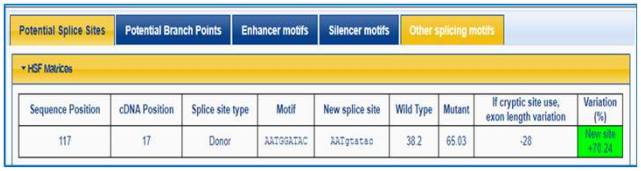


Figure 4. Electropherogram obtained from direct nucleotide sequencing of *F8* exon 14 (forward primer) showing c.4804delC p.(Gln1602Lysfs*19) variant.





Discussion:

In the three patients with severe HMA, three different novel *F8* variants were identified: c.1060_1061delCT, p.(Leu354Thrfs*5) (Fig. 2), c.3561dupT, p.(Pro1188Serfs*10) (Fig. 3) and c.4804delC, p.(Gln1602Lysfs*19) (Fig. 4). All these variants create a frameshift, leading to a premature termination codon and presumably resulting in non-functional truncated proteins, confirming the patient's phenotypes.

The novel *F8* missense variant c.5836G>T, p.(Asp1946Tyr) (Fig. 1) was identified in two unrelated patients, both with mild HMA. The Asp1946 is a highly conserved amino acid in the FVIII protein. Additionally, physicochemical properties between Asp and Tyr are significantly different (while Asp is a small, negatively charged, and polar, Tyr is an hydrophobic, aromatic amino acid) and *in silico* analysis classified it as pathogenic due to the amino acid substitution. According to *in silico* analysis, this variant can also disturb the normal mRNA splicing process due to the creation of a new donor splice site (Figure 5). RNA studies and other functional assays are essential in order to establish this variant clinical significance.

Identification of novel pathogenic *F8* variants in HMA patients allows genotype-phenotype correlations, appropriate genetic counseling and new knowledge about the molecular bases of this pathology.

REFERENCES:
1- Factor VIII variant database - http://www.eahad-db.org; 2- Alamut Visual - alamut.interactive-biosoftware.com; 3- Varsome The Human Genomics Community - https://varsome.com
4- http://www.hgvs.org/mutnomen; 5-Ensembl Variant Effect Predictor (VEP) -https://www.ensembl.org › vep; 6-Human Splicing Finder - Version 3.1 - www.umd.be